LABORATORY STUDIES ON THE INFLUENCE OF MALES ON REPRODUCTIVE ACTIVATION IN FEMALE MONTANE VOLES (*MICROTUS MONTANUS*)

BY

D. KIM SAWREY

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Abstract of Dissertation Presented to the Graduate School
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In a first series of studies, the influences of conspecifics on the reproductive physiology of female montane voles (Microtus montanus), a species exhibiting induced ovulation, were examined. Vaginal smears contained more cornified cells when females were housed in a room also containing males than when only females were present. Housing a male across a wire mesh barrier from a female produced a transient increase in the proportion of cornified cells present in vaginal smears. Housing females from weaning with an adult male accelerated two measures of reproductive activation: vaginal opening and first appearance of cornified smears. When placed together, many sexually inexperienced pairs copulated within 12-24 h.

In a second series of studies, the reactions of both sexes to bedding materials soiled by conspecifics were measured. In a simultaneous two-choice situation, sexually-inexperienced adult females strongly preferred bedding soiled by an adult male to either clean bedding or bedding soiled by a female. Sexually inexperienced males were relatively indiscriminate. After extensive monogamous sexual experience males exhibited a preference for bedding soiled by a female rather than clean bedding.

These results are compared to relevant reports from other Microtus species. Emphasis is placed on rodent chemosignals as important stimuli for sexual selection as well as on the active role of the female in mate choice based on these signals.

GENERAL INTRODUCTION

The roles played by male mammals in reproduction are becoming increasingly appreciated. Until recently, many viewed the provisioning of the female with sperm as virtually the only contribution made by males to reproduction. In recent decades research with rodents has increased our understanding of the variety of stimuli from males that have effects on reproductive function in females. Of particular recent interest are changes in female reproductive physiology that occur as a result of contact with chemosignals from conspecific males (Brown & Macdonald, 1985; Doty, 1976; Vandenbergh, 1983).

However, even in light of the important physiological changes that occur in females after contact with male chemosignals, little research emphasizes the potential active role of females in controlling their exposure to these theriogenic agents. Particularly among those mammals for which stimuli from males are prerequisite to receptivity and ovulation, it would seem that active approach to males or their chemosignals represent important instances of female choice. Yet studies of the olfactory preferences of males, rather than females continue to be overrepresented in the literature.

In the first half of this dissertation studies are reported in which I investigated the influences of male montane voles (Microtus montanus) on conspecific females. The first three studies were designed as initial attempts to quantify the influence of male stimuli on changes in vaginal cytology. In subsequent experiments I examined the amount of male exposure required to induce sexual receptivity and accelerate puberty.

Given that males do influence reproductive physiology in females, a question remains as to how this influence is accomplished. How are the sexes brought together? The second half of this dissertation consists of a series of experiments designed to investigate the attractivity of deposited chemosignals to female and male montane voles.

Male Influences on Rodent Reproduction

In many rodent species, if an unfamiliar male is introduced into a cage housing a pregnant female, the likelihood of pregnancy termination increases. This disruption of pregnancy, originally demonstrated for Mus musculus (Bruce, 1959, 1960), has been shown to occur in a variety of rodent species and at a variety of times during pregnancy. The Bruce effect, as it is frequently called, is not dependent on the presence of the unfamiliar male, but rather is mediated by chemosignals in male urine (Dominic, 1965). The Bruce effect thus represents a situation in which male chemosignals appear to

have an important impact on the lifetime reproductive success of the animals involved (Schwagmeyer, 1979).

Another aspect of rodent reproduction that is subject to the influence of chemosignals from conspecifics involves the acceleration of reproductive maturation. Research with Mus musculus has provided evidence of at least four separate chemosignals that influence puberty in young female house mice. The first of these to be investigated, and the one that is most relevant for this dissertation, involves an acceleration of female puberty by chemosignals present in the urine of males (Vandenbergh, 1967). As is the case with the Bruce effect, puberty acceleration is generally thought to influence reproductive success, in this case by decreasing the age at which females first reproduce (Drickamer, 1986).

Among rodents that experience estrous cycles, male chemosignals have been shown to reduce the variability of and synchronize the cycles of group housed females (Whitten, 1956). Again this effect is mediated by chemical stimuli originating from males and again the suggestion has been made that this is a selected response that increases the fitness of the animals involved.

Chemosignals and Induced Ovulators

Members of the genus Microtus that have been investigated to date lack the regular estrous cycles typical of laboratory

rats (Rattus norvegicus) and mice (Mus musculus). As induced ovulators, they require external stimuli for the stimulation of ovulation. The effective stimuli for the induction of ovulation originate from conspecific males (Richmond & Stehn, 1976; Sawrey & Dewsbury, 1985). Copulation generally provides the most potent stimuli for ovulation, but females of some species can be induced to ovulate in response to more limited contact with males, and in some instances ovulation can occur after exposure to chemosignals from males (Sawrey & Dewsbury, 1985).

A correlate of this pattern of induced ovulation is that sexual receptivity in Microtus females also is made more likely by stimuli from males (Richmond & Stehn, 1976; Sawrey & Dewsbury, 1985). The exact nature of the stimuli required and the duration of exposure necessary to result in sexual receptivity appears to vary among species in the genus.

The relationship between cell types in the vaginal epithelium and behavioral receptivity in Microtus is not well understood. It is frequently assumed that females separated from males will exhibit vaginal smears dominated by leukocytes until exposed to some type of male stimulation. After exposure to a male, cornified smears, presumably indicative of sexual receptivity, are assumed to appear (Seabloom, 1985). The available data for Microtus species suggest that this description is certainly not typical of all members of the genus and may actually occur only rarely in the genus as a whole (Sawrey & Dewsbury, 1985).

Chemosignals as Sex Attractants

In olfactory preference tests both male and female rats (Rattus norvegicus) prefer to investigate heterosexual rather than homosexual urine (Brown, 1977). Both males and females of many species distribute theriogenic substances in the environment that include information regarding the sex of the donor. This at least hints that chemosignals might function as sex attractants for some rodents.

Much interest has been focused on the abilities of males to discriminate between estrous and diestrous odors of female rodents. For a number of species including desert wood rats (Fleming, Chee, & Vaccarino, 1981), brown and collared lemmings (Huck & Banks, 1984), and gerbils (Block, Volpe, & Hayes, 1981), a preference for estrous stimuli has been demonstrated. However, similar attempts with other species have failed to provide evidence of such a preference (Dewsbury, Ferguson, Hodges, & Taylor, 1986; Randall, 1985).

Perhaps as a result of their lack of estrous cycles, very little similar research has been done with Microtus. Taylor and Dewsbury (1988) found that only particular types of sexual and social experience allowed male M. ochrogaster to make an estrous-diestrous discrimination. In an unpublished study, Taylor found that sexually experienced M. montanus males were unable to discriminate between estrous and diestrous caged

unable to discriminate between estrous and diestrous caged females.

Even less research has been done to investigate the possible role of odors from male Microtus as sex attractants for females. In spite of the fact that female Microtus require stimuli from males for the induction of receptivity and ovulation, tests for the preferences of females for male odors apparently have not been reported.

The Biology of Microtus montanus

Montane voles, Microtus montanus, are particularly well suited as subjects for the research reported here. Their behavior and social structure in the field have been relatively well studied (Jannett, 1978, 1980, 1981a, 1982). They survive and breed successfully in captivity and adapt well to various laboratory settings (Dewsbury, 1973; Dewsbury, Lanier, & Miglietta, 1980; Evans, Katz, Olson, & Dewsbury, 1978; Sloane, Shea, Procter, & Dewsbury, 1978; Webster, Williams, Owens, Geiger, & Dewsbury, 1979).

The mating system of M. montanus is generally described as polygamous, but it apparently is flexible, varying to monogamous at with low animal densities or seasonal weather fluctuations (Jannett, 1980). In the field, territories of males overlap the territories of several females, and juveniles sometimes remain together for a period after being abandoned by

the dam (Jannett, 1978). Like other members of the genus, females are induced ovulators, yet little is known about the influence of male conspecifics on reproductive behavior and physiology.

GENERAL METHODS

Subjects and Housing

All subjects were laboratory-bred descendants of a population of Microtus montanus acquired from a colony at Tulane University and long maintained at the University of Florida (Dewsbury, 1973). Efforts were made to avoid inbreeding and maintain genetic diversity. Pairs selected as breeding stock did not have common grandparents. Unless otherwise specified, all subjects were mature adults that had been maintained in litters until used in the experiments reported here. Prior to testing most animals were housed with littermates of the same sex, however, a small minority of animals were housed with other-sex littermates present. Thus, the possible range of sexual experience of the subjects prior to testing was severely limited. Certainly most were virgins, but the possibility of a limited number of sibling-sibling matings cannot be ruled out.

Animals were maintained in a windowless, air-conditioned room that housed the M. montanus colony in the Psychology Department at the University of Florida. A reversed 16:8 light/dark photoperiod, with light onset at 2000 h, was in

effect for all studies. Light from two or three 15-W red bulbs shone constantly. All animals were housed in clear plastic cages with wood shavings as substrate and were provided with Purina rabbit chow and water ad libitum.

Vaginal Smears

Samples of the vaginal epithelium were made with a thin wire loop and tap water, stained with toluidine blue, and examined with a microscope. In studies that involved daily smears, they were made between 800 and 1200 h. Initially smears were scored using the designations estrous, diestrous, and metestrous. These designations proved largely insensitive to many of the relatively subtle changes that occur in this species: a large majority of the smears were classified as estrous, even though percentages of cornified cells fluctuated over a large range. A scoring system more sensitive to these fluctuations was developed and eventually used to score all smears.

All smears scored prior to development of the new system (those for the first four studies of this dissertation) were viewed again and assigned new designations as described below. All other smears (those from the fifth study of this dissertation) were initially scored with the revised system. Several weeks after the completion of all studies involving vaginal smears, all smears were again viewed and scored independently of their previous designation. Thus, all smears

were scored twice using the system described below. On those occasions when the two scores were not in agreement, the smears were viewed again and the total number of cells of each type present in a representative field of view counted.

Actual scoring was accomplished by counting numbers of cells of each type present in a representative portion of a field of view and making an estimate of the total proportions of cells present. The following mutually exclusive designations were used to label smears:

C At least 90% of the vaginal cells present were cornified epithelial cells.

C+ At least 70%, but less than 90%, of the cells present were cornified cells.

C+L Cornified cells and leukocytes were present in roughly equal numbers (each 40-60% of total). Nucleated cells comprised less than 10% of total.

3 Cornified cells, leukocytes, and nucleated cells all present in roughly equal numbers.

L+ At least 70%, but less than 90%, of the cells present were leukocytes.

L At least 90% of the cells present were leukocytes.

Very occasionally nucleated cells appeared in large numbers. When the percentage of nucleated cells was between 40 and 60, the designation C+N and L+N were used. These combinations of cells were present only very rarely (< 1%) and for purposes of data display were combined with smears scored as 3.

Odor Preference Testing

The test arena for the odor preference studies was a 48 x 27 x 13 cm clear plastic cage that had been modified to allow presentation of odor stimuli. A single circular hole was cut at each end of the apparatus to accomodate a small glass jar. Each jar was approximately 4.5 cm in diameter and 8.5 cm deep. The plastic lid of each jar had been altered so that a circular opening approximately 4 cm in diameter could be covered with a fine mesh sceen to enclose stimuli. The screen formed a shallow cone extending approximately 2.5 cm into the jar. The lower edge of the opening of each stimulus jar was situated approximately 3 cm above the substrate.

Stimulus animals were placed in clean 29 x 19 x 13 cm clear plastic cages with wood shavings as bedding. The bedding that served as an odor stimulus was collected after an individual stimulus animal had resided in the cage for 72 h and

was used within 30 min of collection. Soiled bedding was collected from each corner and the center of a cage. Obvious latrine areas were not avoided, thus fecal material and urine were included in every sample. The ad libitum food supplies of odor donors were placed in small jars in an effort to minimize contamination of the bedding by food. Any visible particles of food were removed from stimulus bedding.

All odor preference testing occurred in the M. montanus colony room during the dark phase of the photoperiod between 1300 and 1800 h. Subjects were introduced into the apparatus and allowed 15 min to habituate. Empty stimulus jars were in place during the habituation period. These empty jars were then removed and the stimulus jars containing bedding inserted into the ends of the cage and a 10-min testing period initiated. An Esterline-Angus event recorder was used to record the amount of time spent in each half of the apparatus as well as the time spent scratching or gnawing the wire screen covering the bedding stimuli. Positions of the stimuli types were reversed after each test. Stimulus jars were washed with a commercial detergent, thoroughly rinsed, and allowed to air dry after use. San-i-cel was used as substrate in the test apparatus and replaced after each test.

Vaginal Smears in the Colony Room

The first study was designed to provide baseline data regarding vaginal smears collected in the colony room. Females housed alone, away from direct contact with males, were thought to represent an appropriate group against which the influences of males could be measured.

Previous published reports concerning vaginal smears in M. montanus have provided incomplete information regarding the procedures employed and/or the results obtained (Gray, Davis, Zerylnick, & Dewsbury, 1974; Jannett, 1980; Negus & Pinter, 1966). Vaginal smears were made in preparation for or in addition to the data of primary concern and as a result were not adequately described. In addition, direct comparisons among studies are made difficult by the idiosyncratic scoring systems employed to report vaginal smear data.

In a previous study from this laboratory, Gray et al.(1974) reported that M. montanus females "characteristically showed a predominance of cornified cells (50% or more) for periods of days alternating with several days of leukocyte

invasion. Nucleated cells were rarely dominant."(p.195) A slightly different picture emerges from the vaginal smear data discussed by Jannett (1980). During a 12-day observation period Jannett found that 15 of 25 females failed to exhibit a "cell pattern of > 50% cornified and/or nucleated" cells. Negus and Pinter (1966) examined vaginal smears of 10 animals and reported that the "females with one exception, were not in estrus throughout" the 10-day study period.

These three studies suggest that there is a lack of agreement as to the type of vaginal smear most frequently found in M. montanus. The present study was designed to resolve the apparent inconsistencies in the above mentioned papers as well as to provide baseline data for comparison with those collected in later studies.

Method

The subjects for this experiment were 14 adult M. montanus aged 70-100 days at the beginning of the study. Each female was removed from her littermate group and housed individually in a 29 x 19 x 13 cm clear plastic cage. These cages were placed on a rack housing numerous groups of animals of both sexes in the M. montanus colony room. Vaginal smears were made daily for 30 days and scored according to the procedure described in the General Methods section. This study

began on the last day of January and ran throughout the ensuing February.

Results

Smears dominated by cornified cells (i.e., containing at least 70% cornified cells) were relatively numerous, accounting for 29.8% of all smears (Figure 1; striped bars). By comparison, leukocytes were the dominant cell type in only 2.2% of all smears. However, the most commonly observed smear type was one containing both large numbers of cornified cells and large numbers of leukocytes (C+L = 58.4%). Nucleated cells were never the dominant cell type and were present in significant numbers only infrequently, and then usually in combination with significant numbers of both cornified cells and leukocytes (i.e., smears scored as 3 = 9.6%). Thus, cornified cells were present in fairly large numbers (i.e., >30% of total cells) in 97.8% of all smears.

No evidence of regular, cyclical changes in vaginal cell composition was seen in any of the 14 subjects (Appendix). Changes in the proportions of cell types present over time occurred gradually and aperiodically. An individual female frequently would exhibit the most common smear type (large numbers of both cornified cells and leukocytes) for several days followed by a gradual shift in the percentages of cornified cells and leukocytes. Generally, these increases in the

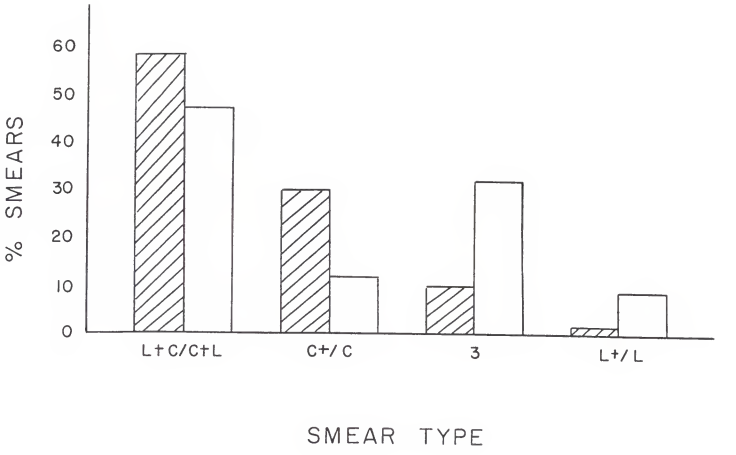

FIGURE 1. PERCENTAGE OF VAGINAL SMEAR TYPES FOR ANIMALS HOUSED IN COLONY ROOM VERSUS OUTSIDE COLONY ROOM. STRIPED BARS REPRESENT ANIMALS HOUSED IN COLONY ROOM. OPEN BARS REPRESENT ANIMALS ISOLATED FROM COLONY ROOM.

proportion of either cornified cells or leukocytes were relatively brief (1-3 days) and were followed by another variable period of time during which again large percentages of both cornified cells and leukocytes were present.

Discussion

Under the current testing conditions cornified cells were rarely absent from the vaginal smears of M. montanus. In most smears (97.8%), cornified cells accounted for at least 30% of total cells. Leukocytes were rarely dominant but frequently appeared in large numbers with cornified cells. The regular, cyclical fluctuations of vaginal cytology typical of spontaneously ovulating rodents was not observed.

The data reported here appear to be fairly similar to those discussed by Gray et al. (1974) for 4- to 6-month-old virgins. They referred to periods of days with a predominance of cornified cells that were followed by shorter periods of leukocyte invasion. Gray et al. did not estimate numbers of leukocytes present during "invasion" nor were leukocytes referred to as the dominant cell type. When the numbers of cornified cells in a smear make them the dominant cell type, their large size enhances the difference in number. When leukocytes invade, their reappearance in substantial numbers is dramatic. In the present study, although the percentage of leukocytes apparent could quickly rise to 40-50, smears with

more than 70% leukocytes were relatively rare. This suggests that the periods of leukocyte invasion referred to by Gray et al. were probably not always periods of leukocyte domination, but at least some of the time, periods characterized by increased numbers of leukocytes from previously low levels. In the present study smears dominated by leukocytes (i.e., leukocytes > 70%) were rarely observed, and never observed on consecutive days. This apparent discrepancy between the present results and those of Gray et al. is likely the result of the different smear scoring systems utilized.

Comparisons with reports from other laboratories reveal differences that are not as easily resolved. Both Jannett (1980) and Negus and Pinter (1966) reported relatively lower frequencies of "estrous" smears than found in the present study. Jannett examined the smears of 25 virgin M. montanus (age unspecified) housed in groups of four or five individuals. After three weeks of group housing, daily smears were taken for 12 consecutive days. During this twelve-day period 15 of the 25 females failed to exhibit a "cell pattern of > 50% cornified and/or nucleated cells." It is possible that this low proportion of females with smears dominated by cornified cells was at least partially the result of the earlier group housing regime. It is well known that various aspects of reproductive function may be inhibited when female rodents are group housed (van der Lee & Boot, 1955). If these were young females, they may have been particularly susceptible to the effects of group housing.

Negus and Pinter (1966) did use young animals; the 10 females were between three and four weeks old at the beginning of the study. After an initial week of smears (during which "no active estrous cycles" were found; p. 598) nine of ten females did not exhibit an estrous smear throughout the following 10-day period. Unfortunately, details of the cellular composition of the smears were not published. Differences between the Negus and Pinter study and the present study in the relative frequency of smears dominated by cornified cells might be due to differences in the ages of the subjects (see Petersen, 1986), diets, or other unspecified housing variables.

Vaginal Smears Outside the Colony Room

Microtus females are known to be sensitive to conspecific chemosignals. One possible reason for the discrepant findings discussed above involves the variability in standard housing conditions among different laboratories. The possible variations in ambient odor stimuli available to animals under different conditions could cause differences in vaginal smear patterns. Unfortunately details concerning the number, age, sex, and kinship of cagemates are often not reported. Even more frequently the number and proximity of other conspecifics in the testing and colony rooms are not reported.

The previous study was conducted in a room housing many (>200) M. montanus of all age-sex classes. The rack housing the

experimental animals also housed numerous other individually caged animals of both sexes; an adjacent rack housed breeding pairs and their unweaned litters; a second adjacent rack housed mainly weaned litters of various ages and sex ratios. The second study was designed to measure the possible effects of removal from the colony room on the smear patterns found among individually housed females.

Method

Subjects for this study were six mature female *M. montanus* (120-140 days). The animals were removed from littermate groups and housed individually in 29 x 19 x 13 cm clear plastic cages. This group of females was removed from the general colony room and placed in a separate room approximately 10 m distant in which no other animals were housed. Thus, except for stimuli from the six subjects, conspecific visual stimuli were eliminated, conspecific auditory stimuli presumably entirely eliminated, and conspecific olfactory stimuli at least greatly reduced. Daily vaginal smears were taken for 30 consecutive days in February and March.

Results

As was the case for females housed in the colony room, no regular, cyclical fluctuations in smear patterns were found in

females isolated from the colony room. Two major differences were noted between smears made in the colony room and those made removed from it. First, the proportion of smears dominated by cornified cells (> 70% cornified cells) was considerably less among animals removed from the colony room than among females remaining in the colony room (11.9% vs. 29.8%). Second, nucleated cells were more common in the smears taken outside of the colony room, but they were still never the dominant cell type. This trend is reflected in the percentages of smears scored as 3 in the two conditions. In the colony room 9.6% of smears received this designation, compared to 31.6% of smears outside the colony room (see Figure 1).

Discussion

The results of this study suggest that there are influences on female reproductive function that are the result of the large number of animals frequently housed in colony rooms. While the modality of the relevant stimuli that caused the differences found in the present studies cannot be determined with certainty, given the known role of olfactory cues in female reproductive function among Microtus, the differences in ambient conspecific odors in the two testing situations are likely to be involved.

Regardless of the exact nature of the cause of the differences in smear patterns seen, there are implications for

research conducted with animals that spend at least the majority of their day in rooms with high densities of conspecifics. Certainly, when vaginal smears are used as a dependent measure, investigators need to be alert to the possibility that stimuli from other nearby colony members may influence results.

Typical conspecific densities found in many laboratory colony rooms almost certainly exceed those frequently present in the field. Laboratory reports designed to measure variables influenced by conspecific odors should include descriptions of animal densities in animal housing and testing areas. Facilities with relatively low animal densities may be desirable or even necessary for such studies.

Vaginal Smears and Proximity to a Single Male

Chemosignals contained in male urine are presumed to mediate the increase in serum estrogen seen in M. ochrogaster females after exposure to males (Carter, Getz, & Cohen-Parsons, 1986). With increasing serum estrogen levels, several other indications of reproductive activation can be measured. In M. ochrogaster various estrogen-sensitive tissues, including the ovaries and the uterus, respond to male chemosignals (Carter, Getz, Gavish, McDermott, & Arnold, 1980; Carter, Witt, Schneider, Harris, & Volkening, 1987). In particular the vaginal epithelium is exquisitely sensitive, responding rapidly with increasing cornification from 48 to 72 h after exposure to males (Hasler & Conaway, 1973; also Richmond & Conaway, 1969 a & b). In addition

to M. ochrogaster, male proximity without direct contact has proven to be an effective stimulus for increasing the percentage of smears dominated by cornified cells in a number of other Microtus species including M. pennsylvanicus (Baddaloo & Clulow, 1981), M. pinetorum (Schadler & Butterstein, 1979), M. californicus (Kenney, Hartung, & Dewsbury, 1979), and M. longicaudus (Jannett, 1982).

Gray et al. (1974) reported that female M. montanus housed across a wire mesh barrier from reproductively mature males "showed a tendency to more days of cornified predominance" but provided no additional relevant data. Jannett (1980) found that of eight females that did not exhibit a cornified smear for twelve days when housed with four or five females, six showed smears with >50% cornified and/or nucleated cells after pairing with a male. Thus there are suggestions that male chemosignals might increase the likelihood of vaginal smears dominated by cornified cells. This study was designed to examine the time course of any vaginal epithelial changes that might occur in response to male proximity with only very limited possibilities for physical contact.

Method

Subjects were 24 mature female (120-170 days) and 24 mature male (150-200 days) M. montanus. Females were removed from their littermate groups and placed in one half of a 48 x 27

x 13 cm clear plastic cage that was divided into two equal areas by a wire mesh barrier. Daily vaginal smears were initiated and continued for 14 days. Males were introduced into the cages across the mesh barrier from females immediately after smears were taken for day 10 and smears continued for an additional four days. The openings of the mesh were small enough to prevent extensive behavioral interactions but a variety of chemosignals could easily have been passed across the barriers.

Data for 12 of the 24 pairs were collected in July and for the remaining 12 pairs in November. The two sets of smears collected before the introduction of males were compared in an effort to detect seasonal changes in cell types present.

Results

After introduction of the males there was an increase in the number of females with smears dominated by cornified cells (C and C+), as well as a general increase in the proportion of cornified cells in smears (see Figure 2). This increase in cornification was transient, lasting about two days. A comparison of smears taken just prior to introduction of the males with those taken after 24 h of male exposure revealed that 14 of 24 females exhibited an increased proportion of cornified cells, 3 females showed decreased cornification, while 7 exhibited no measured change in cell-type proportions with the

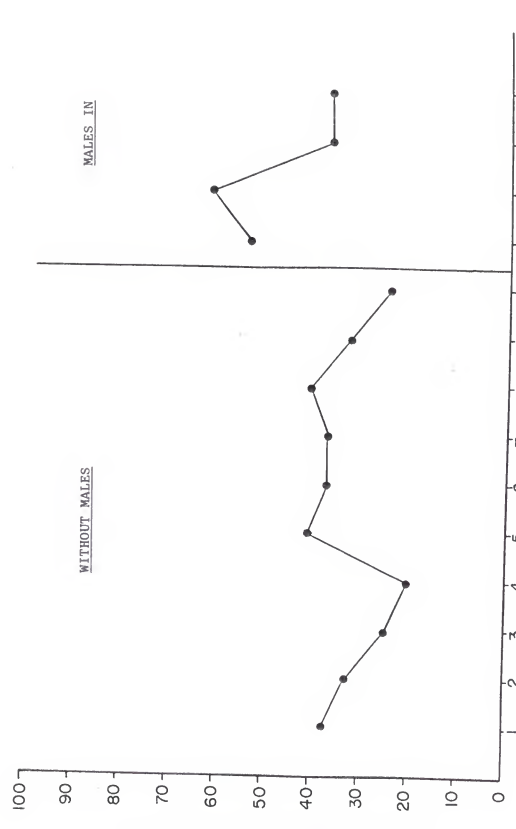

FIGURE 2. MALE EFFECTS ON MONTANE VOLE SMEARS

scoring system used. This measure of increased cornification was statistically significant (p = .012, two-tailed binomial test).

Figure 2 shows the percentage of females with smears dominated by cornified cells on each day of the entire duration of the 14-day study period. More females showed cornified smears on the two days following male introduction than on any other day of the study.

The percentages of the various smear types present in July and November were very similar. For this analysis smears dominated by cornified cells were considered together in one category (i.e., C and C+), as were smears that included large numbers of leukocytes or nucleated cells (i.e., L, L+, and 3), while smears with large numbers of both leukocytes and cornified cells (i.e., C+L) were considered as a separate category. The percentages of smear types in July and November for cornified smears (33% vs. 35%), smears dominated by leukocytes (31% vs. 20%) and C+L smears (49% vs. 57%) showed little seasonal variation. The numbers of individuals exhibiting increased cornification was the same for both months (7).

Discussion

Even though large numbers of cornified cells are usually present in vaginal smears of *M. montanus*, placing a male adjacent to a mature female frequently increases the percentage of cornified cells present. Thus, at least one measure of

reproductive activation reflects a role for male proximity in the initiation of the reproductive sequence. As in the previous study the dimension(s) of the relevant stimuli cannot be definitively ascertained.

The response to male proximity found in this study suggests that the increase in cornification occurs quickly and lasts only briefly. This quick, brief response does not seem to be a general pattern among Microtus. Richmond and Conaway (1969a) found no cornified cells in female M. ochrogaster housed next to males until after at least 48 h of exposure. They reported that only 37.5% of females thus exposed were receptive or had attained an estrous smear by the end of three days of treatment. However, once estrous smears did appear, Richmond and Conaway reported that the most common pattern was that of persistent cornification lasting at least several days or until the animal was mated. The influence of male proximity was considerably shorter lived with the M. montanus used in this study.

Latency of Naive Pairs to Initiate Copulation

The results of the previous experiment suggest that stimuli from males rapidly initiate physiological events that may culminate in reproductive activation. The appearance of increased vaginal cornification, the measure of reproductive activation examined above, occurred quickly, within 24 to 48 h of male introduction. This study was designed to determine the time course

of subsequent elements of reproductive activation in M. montanus. Specifically, the latency to initiate copulation after pairing was investigated.

Dewsbury (1981) proposed that differences in the latency to initiate copulation might be correlated with differences in mating systems among rodents. In particular, monogamous pairs are expected to spend relatively long periods of time in courtship, evaluating potential exclusive mating partners. This proposal was made in relation to short-term behavioral tests in which females were reproductively primed either with hormone injections or exposure to a male. Given the role of stimuli from the male in reproductive activation in Microtus, the total time from pairing to copulation without prior reproductive priming might serve as a relevant measure of "latency to initiate copulation" for comparison of Microtus species. When placed together, sexually naive pairs of M. ochrogaster typically initiate copulation between 24 and 72 h later (Carter et al., 1986; Witt, Carter, Carlstead, & Read, 1988). Extending the role of rapid reproductive activation found in the previous study, it was predicted that M. montanus pairs would begin sexual activity sooner after pairing than pairs of M. ochrogaster.

Method

Subjects were 18 adult female (150-210 days) and 18 adult male M. montanus. Females were removed from their littermate groups and placed in 48 x 27 x 13 cm clear plastic cages. Daily vaginal smears were taken for three days. After vaginal smears had been made at 1000 h on day three, a single male was introduced into each cage. Vaginal smears were taken again after 12 h at 2200 h. On each of the subsequent four days smears were taken at three times each day: 1000 h, 1600 h, and 2200 h. After the fourth day of this regime, daily smears were resumed for the duration of the experiment. The presence of sperm in smears and the date of birth of all litters were also recorded.

Results

Sperm were found in the first post-pairing smear made in 5 of 18 cases. Thus, at least 27.8% of the pairs had copulated within 12 h of pairing. Sperm appeared, for three additional females, in smears made after an additional 12 hours. Based on the 21-day gestation period of M. montanus (Negus & Pinter, 1965) and the date of appearance of litters for two additional pairs, a total of 10 of the 18 pairs copulated during the first 24 h after pairing. Of the remaining eight pairs, sperm appeared between 48 h and 15 d after pairing in three cases, while no

evidence of copulation was detected in the remaining five cases (smears in these instances were continued after pairing for 12 days, N=3, or 25 days, N=2).

Discussion

A majority of pairs of M. montanus initiated copulation within 24 h of being placed together in the laboratory. Positive evidence of copulation was found for 13 of the 18 pairs of animals in this experiment. For 10 of these 13 pairs, copulation was initiated within 24 h of pairing.

The available data for M. ochrogaster suggest very different timing for the initiation of copulation. Time-lapse videotape records provided no evidence for the occurrence of copulatory behavior in the first 24 h after pairing regardless of whether sexually naive females were placed with naive or sexually experienced males (Carter at al., 1986; Witt et al., 1988). Similarly long latencies to copulate in M. ochrogaster have also been measured in this laboratory using sexually experienced males.

Combined with the data from the previous experiment, it appears that the responses of sexually naive females to males are more rapid in M. montanus than in M. ochrogaster. It is possible that this measure of latency to initiate copulation will prove to be related to mating systems among Microtus. Data from additional species will be required to determine if the

trenad seen here, that species that are frequently monogamous spend more time in courtship than more promiscuous species, is a general one.

Puberty Acceleration of Females by Males

The age at which animals first reproduce has long been recognized as an important parameter influencing population growth as well as individual lifetime reproductive success. The discovery that stimuli from male mice (Mus musculus) accelerate the occurrence of reproductive maturition in females (Vandenbergh, 1967) resulted in an explosion of research (see Drickamer, 1986; Vandenbergh, 1983).

It is now recognized that stimuli from males accelerate various measures of reproductive maturition in a variety of rodent species. In the genus Microtus the influence of males on the attainment of puberty has been demonstrated for several species including M. agrestis (Clarke & Clulow, 1973), M. ochrogaster (Carter et al., 1980; Hasler & Nalbandov, 1974), M. pennsylvanicus (Baddaloo & Clulow, 1981), and M. pinetorum (Lepri & Vandenbergh, 1986). For all of these species, physical contact with a male causes early reproductive activation in females relative to females not placed with males. In the two cases for which the relevant data are available (M.ochrogaster and M. pinetorum) a behavioral/tactile cue from the male appears to be required for complete acceleration.

The present experiment was intended to measure any accelerative effect of males on reproductive functioning in M. montanus females. Additionally, an effort was made to determine if such an effect required extensive physical contact with males.

Method

Females were removed from family groups at 18 days of age and placed into one of three experimental situations. Fourteen female weanlings were each placed in a 48 x 27 x 13 cm plastic cage with an adult male (Group 1). Fourteen additional females were each placed across a wire-mesh barrier from an adult male in one half of a 48 x 27 x 13 cm plastic cage (Group 2), while a final group of 14 females were each placed individually in 48 x 27 x 13 cm plastic cages without a male present (Group 3). All females were inspected daily for evidence of vaginal opening. This was accomplished by gently drawing a moistened wire-loop probe across the site of the vaginal opening. For females with open vaginas a daily vaginal smear was made. After 20 days of this regime an adult male was placed with each of the Group 3 females (now 38 days of age). Vaginal inspection and smears were continued until all animals had exhibited a smear dominated by cornified cells (>70%). Statistical comparisons were made using the Mann-Whitney U test (Siegel, 1956).

Results

At day 18 vaginal opening was apparent in only 5 of 42 experimental subjects. Of these five only a single female exhibited a cornified smear. Vaginal opening had occurred in all animals by day 42 and all had exhibited at least a single smear dominated by cornified cells by day 56.

Vaginal opening occurred at an earlier age for females paired with a male from weaning than for those first paired with a male at day 38 ($U = 43.5$, $p<.01$). The average age of vaginal opening for females housed across a wire-mesh barrier from a male from day 18 was intermediate to the ages for the other two groups, but not significantly different from either (see Table 1).

The first appearance of cornified smears occurred earlier when males were present from day 18 than when introduced on day 38 ($U = 35.0$, $p<.001$). Again values for females housed across a wire-mesh barrier from males were intermediate and not significantly different from the values for the other two groups (see Table 1).

Cornified smears appeared in all Group 1 females and in 11 of 14 Group 2 females by day 38. At the same age only 4 of 14 Group 3 females showed a cornified smear. The responses of the remaining Group 3 females to the introduction of a male were notably regular. Cornified smears made their first appearance in

Table 1

Female Puberty Acceleration by Males

	Mean age (days)	
Group	Vagina open	Cornified smear
1. With male	22.6 a	26.5 b
2. Across from male	25.2	33.7
3. Without male	30.1 a	36.5 b

Note. Means with shared subscript are significantly different ($a = p<.01$; $b = p<.001$).

all of these females between 48 h and 96 h after male introduction.

Discussion

These results clearly indicate that male montane voles provide stimuli that accelerate reproductive maturation in females. Vaginal opening and the first vaginal smear dominated by cornified cells both occurred earlier in females housed with a male than in females housed without males present. For females housed across a barrier from a male, both measures fell between the ages for the other two groups.

The intermediate values found in the group that was housed across a barrier from males represent a potentially interesting finding. This result is at least suggestive of a role for male chemosignals in the acceleration of puberty in *M. montanus*. However, these data do not allow determination of the cause of the apparently more powerful effects of direct male presence. It could have been that the wire mesh did not allow females as much direct contact with male urine (or other accelerating substances) as is necessary for greater acceleration of puberty. Alternatively, it may be that the effect was the result of the limited male-female contact possible through the barrier.

In M. ochrogaster it has been demonstrated that exposure to a male causes increases in estrogen binding in brain as well as increases in serum estrogen in adult (50 days) females (Cohen-Parsons & Carter, 1987). It would seem most parsimonious to assume that the mechanisms underlying adult female reproductive activation and female puberty acceleration in Microtus are the same. In both cases assays of estrogen sensitive tissues exhibit changes predictably following male exposure. Weanling female M. montanus respond to males somewhat more slowly than do adult females, but this may simply reflect the relative immaturity of the brain-gonad axis (e.g., Westlin, 1982).

RESPONSES TO CONSPECIFIC ODORS

Many rodent species possess anatomical and behavioral adaptations that suggest an important role for chemical signals in their social interactions. In general, rodents have well developed olfactory epithelia and vomeronasal organs as well as relatively large brain olfactory lobes. In addition to urine and feces, various specialized glandular tissues are usually present that function to introduce internally manufactured chemical substances into the environment (Quay, 1968; Jannett, 1986). Numerous types of behavior adapted for deposition of these theriogenic substances occur. In virtually all social interactions, behavior patterns that appear to maximize contact with the specialized substances are seen (Eisenberg, 1962, 1967).

For a variety of rodent species, chemical cues have been shown to be instrumental in the timing of puberty, in the occurrence of female receptivity, in the maintenance of pregnancy, and in the appearance of aggressive behaviors (see Brown, 1985; Vandenbergh, 1983). Given these well documented roles of chemical stimuli, it is perhaps not surprising that investigators frequently assume that rodents can easily utilize these stimuli to discriminate among animals based on their sex. Interestingly, relatively few

tests have been made to examine this assertion. Reviews of rodent olfactory studies show that among the myriad of two-choice intraspecific odor preference possibilities (Schultz & Tapp, 1973), the most frequently investigated combination is male choice between odors from receptive and nonreceptive females (Brown, 1977, 1985; Schultz & Tapp, 1973). Certainly it has been demonstrated that several species prefer conspecific hetero- to homo-sexual odors. However, it is by no means an inevitable finding (see Brown, 1985).

Microtus montanus possess a rich variety of possible sources of chemical stimuli (Jannett,1986). Territorial males deposit urine and feces on prominent objects in their environments (Jannett, 1980). Males also exhibit stereotyped behavior patterns, Scooting and Anal Drag, that appear adapted to deposit anal gland secretions on the substrate (Jannett, 1980). Posterolateral scent glands have been shown to direct the attacks of other males (Jannett, 1981b). Glands associated with the eyes, preputial glands, saliva and other substances emanating from the oral region, and vaginal secretions represent other possible sources of relevant theriogenic stimuli.

In various combinations these odor sources are thought to mediate several aspects of the biology of M. montanus. As mentioned above, Jannett (1981b) showed that the posterolateral scent glands of males function as the targets of aggressive behaviors from other males. Various investigators have found suggestive roles for chemical stimuli from conspecifics in female

reproductive activation (Gray et al.,1974; also see above), acceleration of female puberty (see above), and male-induced pregnancy termination (Jannett, 1980; Stehn & Jannett, 1981). Given the apparent sophistication of the olfactory abilities of M. montanus it is not surprising that it has frequently been suggested that chemical cues provide the basis for discrimination of species, sex, and reproductive condition (Brown, 1985; Quay, 1968). It is somewhat surprising that no direct tests of these possibilities exist for M. montanus in the literature. The following experiments were performed to provide baseline information about the responses of M. montanus to conspecific odors.

Female Responses to Male-soiled and Fresh Bedding

Method

Sixteen adult females were tested in the odor preference apparatus with one jar containing fresh wood shavings and one jar containing the same type of bedding that had been soiled by a male. The female subjects were all adults, ranging in age from 60 to 120 days. These females were housed with littermates of the same sex until being individually housed in 29 x 19 x 13 cm clear plastic cages approximateley 10 days prior to the start of testing. The males that served as odor donors were all sexually

mature, ranging in age from 70 to 185 days, and each used no more than once per experiment.

Results

Females spent more time on the side of the apparatus housing the male-soiled bedding than on the side with fresh bedding (see Table 2, row A). The time spent scratching and/or gnawing at the openings of the stimulus jars appeared to be an even more sensitive measure of preference. Females spent an average of 158 s scratching/gnawing the screen covering the soiled and an average of only 13 s manipulating the screen covering the fresh bedding (Table 3, row A). Scratch/gnaw durations at the soiled stimulus exceeded those at the fresh stimulus for 14 of the 16 subjects ($p = .004$, sign test).

Each female made numerous visits to each side of the apparatus and all subjects investigated the openings of the stimulus jars multiple times. Investigations of the fresh bedding were generally brief and included little or no scratching/gnawing while investigations of the male-soiled bedding were of longer duration and consisted most frequently of vigorous manipulation of the screen covering the odor source.

Table 2

Odor Choice Tests: Side Preference

	Animals Choosing		Stimulus Bedding		Mean Time (Sec.)		Mean Preference		Number Animals With Preference	
	Sex	N	Side A	Side B	Side A	Side B	Side A	t-test	Side A	sign test (p)
A	F	16	Male	Clean	365	224	62%	2.66*	13	.022
B	F	16	Male	Female	396	197	67%	3.95**	11	.210
C	M	16	Female	Male	238	356	40%	3.30**	4	.076
D	M	16	Female	Clean	280	311	47%	0.43	5	.210
E	M (Ex)	8	Female	Clean	308	276	53%	0.36	6	.290

Note. Ex = sexually experienced males . * $p < .02$. ** $p < .01$.

Table 3

Odor Choice Tests: Scratch/Gnaw

	Animals Choosing		Stimulus Bedding		Mean Time (sec.) Scratch/Gnaw		Mean Preference		Number Animals With Preference	
	Sex	N	Side A	Side B	Side A	Side B	Side A	t-test	Side A	sign test (p)
A	F	16	Male	Clean	158	13	92%	6.64**	14	.004
B	F	16	Male	Female	162	22	88%	4.11**	15	.001
C	M	16	Female	Male	28	76	27%	1.97	5	.210
D	M	16	Female	Clean	12	51	19%	1.60	5/14	.424
E	M (Ex)	8	Female	Clean	93	26	78%	3.06*	7	.070

Note. Ex = sexually experienced males. * $p < .02$. ** $p < .01$.

Female Responses to Male-soiled and Female-soiled Bedding

Method

The sixteen female subjects used in the previous study were tested on a second task in this experiment. Two to four days after participating in the previous study, females were presented with a simultaneous choice between a sample of bedding soiled by a male and a sample soiled by a female. Again each odor donor was used only once. The female odor donors were sexually mature and had been individually housed for at least 10 days.

Results

The behavior of the animals was very similar in appearance to that seen in the previous experiment. Subjects spent an average of 67% of the total test time on the side of the apparatus housing the male-soiled bedding (Table 2, row B). An average of 162 s was spent scratching/gnawing the screen covering the male-soiled jar and an average of only 22 s with the female-soiled stimulus (Table 3, row B). For all but one subject, scratch/gnaw durations at the male-soiled stimulus exceeded those at the female-soiled stimulus ($p<.001$, sign test).

Male Responses to Female-soiled and Male-soiled Bedding

Method

The previous two studies clearly demonstrated that the apparatus and method employed were suitable for the demonstration of odor preferences in voles. In the present study sixteen adult males, ranging in age from 70 to 185 days, were given simultaneous access to both male-soiled and female-soiled bedding. The animals that served as odor donors were those used in the previous study. The female odor donors had been individually housed for at least 12 days. Thus, although their vaginal state was not recorded, no effort had been made to facilitate reproductive activation via close proximity to a male.

Results

Neither of the measures considered in this study suggested a preference by males for female-soiled bedding. Twelve of sixteen males spent more time on the side of the apparatus housing the male-soiled bedding than on the side with female-soiled bedding ($p = .076$, sign test). In addition, 11 of 16 males spent more time scratching/gnawing the male-soiled stimulus than the female-soiled stimulus ($p = .210$, sign test).

As a group, these animals spent more time on the male-soiled than the female-soiled side (Table 2, row C). The amount of time spent scratching/gnawing was quite low relative to the time spent by females in the previous two studies (Table 3, row C). The non-significant trend toward a preference for the male stimulus seen here was due largely to the high scores of three subjects.

Male Responses to Female-soiled and Fresh Bedding

Method

With the unexpected results of the previous experiment an investigation of male responses to simultaneously presented female-soiled and fresh bedding appeared warranted. The males that were used in the previous study again served as subjects. The same group of female odor donors used for the previous study were used again. No male was exposed to the bedding of a female that he had encountered in the previous experiment.

Results

Males spent almost equal periods, on average, on each side of the apparatus (Table 2, row D). As in the previous study, the males spent far less total time scratching/gnawing the stimulus jars than did the females in the studies in which

they served as subjects (Table 3, row D). Comparing the average time spent scratching/gnawing the female-soiled stimulus (12 s) and the average time with the clean bedding (51 s) is somewhat misleading; one male scratched at the container with clean bedding for almost the entire test period (449 s). Fourteen of the individual scratch/gnaw scores for female-soiled bedding and eleven of the scores for fresh bedding were less than or equal to 20 s. Of the 14 males displaying durations of scratching/gnawing greater than zero, nine males had longer durations at the clean bedding stimulus ($p = .424$, sign test).

Responses of Sexually Experienced Males to Female Odors

Method

The behavior of the males in the previous two studies was unexpected. It was hypothesized that sexual experience might be neccessary before males would exhibit an attraction to female-soiled bedding (Taylor & Dewsbury, 1988). The subjects for this experiment then, were eight males that had been continuously housed with an adult female and had produced at least two recent litters with her. Thus, this group of subjects had extensive monogamous sexual experience. Female-soiled bedding and fresh bedding were used as stimuli.

Results

The results of this study show that males with extensive monogamous sexual experience can discriminate female-soiled bedding from fresh bedding and prefer to approach female-soiled bedding. Although the times spent on each side of the apparatus by this group were very similar (Table 2, row E), subjects spent more time investigating the female-soiled bedding than the fresh bedding (Table 3, row E). Again the average times spent scraching/gnawing the soiled (93 s) and fresh (26 s) stimuli were low relative to the total time spent scratching/gnawing by females. Only one of the eight males tested spent more time scratching/gnawing the fresh than the soiled bedding ($p = .070$, sign test).

Discussion of Odor Preference Studies

The results of this group of studies demonstrate that sexually inexperienced female Microtus montanus were able to discriminate between the odors of male and female conspecifics and preferred to approach the odors of males. The results for males were more complex. Males that had extensive monogamous social and sexual contact preferred female-soiled bedding to clean bedding, while no such preference was demonstrated by sexually naive males. When these same inexperienced males were

tested with male-soiled and female-soiled bedding, a slight preference for male-soiled bedding emerged.

Females

The finding that females prefer the odors of males to the odors of females or the odor of clean bedding is not surprising. A female preference for male over female odors has been found for several species including Rattus norvegicus (Brown, 1977), Mus musculus (Scott & Pfaff, 1970), Dipodomys agilis and merriami (Daly, Wilson & Behrends, 1980), Neotoma micropus (August, 1978) and Meriones unguiculatus (Dagg & Windsor, 1971). The present results may represent the first demonstration of female attraction to male odors in a rodent species with induced ovulation.

The behavior of the females in these tests was noteworthy. The mean times spent by females actively manipulating the containers holding male bedding were long compared to the times spent by other rodents on similar tasks (Dewsbury et al., 1986; Halpin, 1988). The females' interactions with the male stimuli were also very vigorous and persistent. In most situations in which females would encounter male odors this behavior would almost certainly result in eventual contact with the odor source. It may be that contact with the odors of a male, even in the absence of the animal that deposited the substances, is sufficient to initiate the neuroendocrine reflexes critical for reproduction in this species.

Males

Whereas female M. montanus discriminated and preferred to approach male-soiled bedding, the males tested here were not as strongly attracted to heterosexual odor stimuli. When tested with male-soiled and female-soiled bedding, sexually inexperienced males actually spent more time, on average, on the side of the apparatus housing the male stimuli. In the same test males spent an average of 76 s scratching/gnawing the male stimulus and only 28 s with the female stimulus, a difference that was not statistically significant. The fact that naive males spent more time on the side of the apparatus housing male bedding may be indicative of the importance of these odors for males in the field. For an unmated male, establishing a territory exclusive of other males may be prerequisite to acquiring mates.

Beach (1958) suggested that the reproductive behaviors of male mammals are generally more labile than those of females. Similarly, the abilities of males to display sex-related preferences are flexible and, relative to females, dependent on previous exposure to sexual stimuli (see Brown, 1985). Thus, the finding that after prolonged cohabitation with a single female, male M. montanus exhibited a clear preference for female odors over clean bedding is in general agreement with much previous work. However, as this study did not involve a choice between male and female stimuli the possibility remains that the preference

shown was for species rather than sex. In any case, a discrimination was exhibited that was not apparent in males without prior sexual experience.

Methodological Considerations

These studies were originally intended to provide information that might eventually lead to a more complete understanding of the social behavior of M. montanus. However, before such laboratory-to-field extrapolations are attempted, a number of issues regarding the particular methods employed here must be addressed. It should be emphasized that failure to demonstrate a preference for a stimulus under the conditions employed here does not imply that the animals cannot discriminate among the odors tested. It may very well be that sexually inexperienced males are able to discriminate male from female odors, but that female odors are relatively unattractive for these males.

Although the method of odor presentation utilized has been found to be very effective in allowing preferences to be exhibited (Dewsbury et al., 1986; Johnston, 1981) it may be inappropriate for the demonstration of sexual odor preferences by naive male M. montanus. Dewsbury et al. (1986) provided evidence to suggest that the duration for which bedding was soiled might be a critical variable for the demonstration of some types of preference. Compared to most other studies that collect bedding in the manner described for these studies, the period of female habitation was

relatively long. The possibility remains, however, that a longer period of soiling might have made the relevant cues more salient.

Taylor and Dewsbury (1988) found that estrous-diestrous preferences emerged in male M. ochrogaster only after extensive social experience involving simultaneous exposure to both sexes (see also Dewsbury et al., 1986). Monogamous social experience, similar to that which sharpened the male-female preferences of males in the present work, was not effective at engendering estrous-diestrous preferences in M. ochrogaster. Additional studies to investigate the effects of different regimes of social/sexual experience on various sexual odor preferences appear warranted. In particular, comparative data from species whose mating systems have been relatively well characterized, should prove valuable.

GENERAL DISCUSSION

In a landmark paper, Doty (1974) warned that the roles of females in rodent courtship and copulation were being seriously neglected. I would like to issue what amounts to a second "cry for the liberation of the female rodent." Even though male rodents are far more likely than females to possess specialized organs and behaviors adapted to distribute biologically active materials in the environment, and even though numerous aspects of female reproductive physiology can be altered by contact with these substances, the odor preference literature is still dominated by studies investigating the responses of males to chemosignals from females. Investigators frequently report the results of male-choice preference studies while coincidentally failing to conduct the reciprocal experiments, thereby flying largely in the face of 120 years of evolutionary thinking. The series of studies reported here may serve to reemphasize the female rodent as an active agent in the entire reproductive process.

The Influence of Males

Among Microtus a general pattern of female responsiveness to stimuli from males is emerging. For all species for which data are available, males provide stimuli that produce reproductive activation in females. In many cases the relevant stimuli have been shown to be olfactory in nature. The following description of reproductive activation is presented as a general model for Microtus. Whether or not this model proves to have general applicability among Microtus will be dictated by addition data.

Reproductive activation, as discussed here, involves the following. Stimuli from a male are received and transduced by the vomeronasal organ and main olfactory system (Lepri & Wysocki, 1987). These neural messages initiate neuroendocrine reflexes that result in increasing levels of serum estrogen (Cohen-Parsons & Carter, 1987). Under the influence of estrogen, uterine weight increases, vaginal cornification increases, and sexual receptivity becomes more likely (Carter, Witt, Schneider, Harris, & Volkening, 1987). With the stimulation of copulation, ovulation occurs along with additional endocrine sequelae necessary to support pregnancy (Kenney & Dewsbury, 1977).

It is likely that the responses seen in reproductively immature females to stimuli from males involve activation of essentially the same sequence. The relative rapidity with which

reproductive activation occurs in older females is presumably the result of an increase in sensitivity to male signals that occurs with increasing age.

Interspecific variability in the timing of these events and in the stimuli required for their initiation and completion may eventually be prove to be related to the various mating systems exhibited by different Microtus species.

Results in Comparative Perspective

Vaginal Smears

Interspecific comparisons of vaginal smear patterns among Microtus reveal interesting differences. Data collected in this laboratory for M. ochrogaster and scored using the system described above, indicate that adult females exhibit smears dominated by leukocytes more frequently (19% vs. 2%) and smears dominated by cornified cells less frequently (9% vs. 30%) than individually housed M. montanus. It is worthy of note that cornified smears are reported more frequently for M. montanus and M. pennsylvanicus (Baddaloo & Clulow, 1981) than for M. ochrogaster (Richmond & Conaway, 1969 a & b) and M. pinetorum (Kirkpatrick & Valentine, 1970).

The differences in smear patterns seen in these species may reflect differences in serum estrogen levels or sensitivities of target tissues, which may in turn be related to

presumed differences in mating systems. There is much evidence to suggest that M. pennsylvanicus (Madison, 1980, 1981, 1984) and M. montanus (Jannett, 1978a, 1980, 1981a) do not generally form long-term monogamous relationships. On the other hand, a strong case can be made for frequent monogamy in M. ochrogaster (Getz & Carter, 1980; Getz et al., 1981; Thomas & Birney, 1979) and monogamy appears to be a likely possibility in M. pinetorum (Dewsbury, 1981; Fitzgerald & Madison, 1983; Wolff, 1985). The differences in smear patterns seen among these species may also correlate with latencies to copulate, a factor suggested as being related to mating system (Dewsbury, 1981). When sexually inexperienced adult M. ochrogaster (Carter et al., 1986) or M. pinetorum (Lepri & Vandenbergh, 1986) are paired, no copulatory behavior occurs in the first 24 h. Copulation frequently occurs in the first 12 to 24 h after pairing in M. montanus (this study) and M. pennsylvanicus (Lee et al., 1970). The cornified smears seen in M. montanus and M. pennsylvanicus may be indicative of relatively short courtship durations in these two species.

These data add to a considerable body of comparative laboratory data that represent reliable differences among species that may be correlated with mating systems (Dewsbury, 1987). Microtus montanus females respond with rapid vaginal cornification after exposure to a male and spend relatively little time in courtship. This may prove to be a general pattern among Microtus species that are not monogamous. Monogamous

species for which data are available respond less rapidly to male exposure and have relatively prolonged courtship durations.

Chemosignals

Blaustein (1981) suggested that for many small mammals sexual selection may have resulted in sexual dimorphism in chemosignal production. In M. montanus the specialized glands and odor-depositing behavior of males suggest a role for chemosignals in intra- as well as intersexual selection. As discussed above, attacks are frequently directed toward posterolateral glands in male-male encounters (Jannett, 1981b). Microtus montanus females may select among males based on their odors. In this light it is interesting that sexually naive females, but not sexually naive males, prefer and actively approach heterosexual odor sources (this study). At least in this species it would appear that for males, responding to male odors may be a basic, relatively inflexible behavior pattern. Perhaps establishment and defense of a territory are prerequisite to reproduction. The burden of bringing the sexes together and the timing of various reproductive activities may have fallen to females. Jannett (1986) suggested that when females abandon litters at weaning that they may also be exhibiting mate choice. Data from this dissertation appear consistent with the possibility of female M. montanus making mate choice decisions based at least in part on olfactory cues.

It seems likely that a female M. montanus dispersing into a new area would encounter deposited male chemosignals in advance of actual contact with the male. A female might thus initiate the process of reproductive activation, thereby reducing the total male-female contact time required for reproduction. In a monogamous species (e.g. M. ochrogaster) a more detailed assessment of a potential mate might result in selection for an extended period of male-female contact. Indeed, M. ochrogaster have been shown to be contact-prone compared to M. montanus. When conspecific pairs were placed together for three hours and videotaped during the third hour of contact, pairs of M. montanus spent only about two minutes in contact with each other, while M. ochrogaster pairs associated for almost half of the taping duration (Dewsbury, 1988). For a female M. montanus the important stimuli for reproductive activation may not involve contact with the male, but rather contact with his relevant deposited chemosignals. That soiled bedding can be effective in such activation has recently been demonstrated for M. ochrogaster (Carter et al., 1987) and for Suncus murinus (musk shrews), another induced ovulator (Rissman, 1989). Male odors, rather than the male per se, may be the functionally reinforcing stimuli that are approached by female M. montanus to initiate reproductive activation.

There is some additional evidence to suggest that female Microtus actively approach males and may thus regulate their reproductive activation. Carter et al. (1980) found that female

M. ochrogaster avoid being reproductively activated by siblings by avoiding naso-genital contact. If urine from male siblings is placed on the upper lip of females, reproductive activation is initiated. Certainly in this case females might be thought of as making a mate choice decision; they avoid contact with their brothers (and father) that would initiate reproductive activation.

When 25-day-old M. ochrogaster are placed in a large pen containing unfamiliar adult males, they approach the males and initiate their own reproductive activation (McGuire, 1987). Thus, depending on environmental circumstances, young M. ochrogaster females either approach males and initiate reproductive activation or avoid males and postpone reproduction.

Overview and Final Remarks

The following points briefly describe the findings of this dissertation:

1) Vaginal smears of M. montanus provide no evidence of regular cyclical fluctuations.

2) The composition of vaginal smears is influenced by stimuli from males. Placing males adjacent to females increases the proportion of cornified cells present. The presence of males in the colony room also increases proportions of cornified cells

relative to the proportions seen in females housed away from the colony room.

3) Sexual receptivity in female M. montanus appears to follow exposure to a male more rapidly than in M. ochrogaster. Vaginal smears of individually housed M. montanus contain greater proportions of cornified cells than do smears of M. ochrogaster collected under similar colony conditions.

4) Puberty in female M. montanus can be accelerated by the presence of a male. Both vaginal opening and first cornified smear occur earlier in females paired with males at weaning (18 days) than in females paired with males at day 38. Moreover, the vaginal epithelial response of females first paired with a male at day 38 is very regular.

5) Adult, sexually naive female M. montanus strongly prefer the odor of bedding soiled by males to either female-soiled or clean bedding.

6) Adult, sexually naive male M. montanus show no preference when presented with a choice between female-soiled bedding and clean bedding.

7) The same males exhibit a slight preference for male-soiled bedding when the alternative odor source is female-soiled bedding.

8) Males with extensive monogamous social and sexual experience prefer female-soiled bedding to clean bedding.

These findings, in conjunction with previous studies, begin to provide the information needed to formulate a more comprehensive view of the biology of M. montanus. The picture that emerges is not really a new one, but one that now may be seen in somewhat greater relief.

The field studies of Jannett (1978, 1980, 1981a, 1982) have revealed that males establish territories that they maintain essentially exclusive of other males. At some stage in the life of a male, establishing such a territory appears to be essential for reproductive success. Male-male competition is almost certainly intense; the odors of other males may present a more urgent message than the odor of females. Two males placed in a 4 x 8 ft enclosure with two reproductively mature females continue to show high levels of male-male aggression for at least 5 days (the duration of the study), and exhibit very low levels of sexual behavior (Dewsbury, 1983). It may be that early in the life of a male, before a territory is established, male odors are more important stimuli that female odors.

Once a male has established a territory, resident females come under his olfactory influence. Male M. montanus are equipped with a variety of specialized odor sources and behaviors to disseminate chemical messages throughout their territories. Females are rather exquisitely sensitive to chemical cues from males. It is likely that chemical cues from

males are responsible for the reproductive activation found in the present research. In the field, the effects of exposure to male chemosignals combined with the attractivity of these substances to females, almost certainly result in females actively seeking out prospective mates based on these signals.

APPENDIX
VAGINAL SMEARS IN COLONY ROOM: DATA FOR INDIVIDUAL SUBJECTS

	SUBJECT NUMBER:													
DATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1/31	C+L	C+L	3	3	C+	C	C+	C+	C+	C	C+L	C	C	L+C
2/1	C+L	C	L+C	L+C	L+C	C	L+C	C+	3	C+L	C+	L+C	C+	L+C
2/2	C+L	C+	L+C	L+C	L+C	C	L+C	C	L+C	L+C	L+C	L+C	L+C	C+L
2/3	L+C	L+C	L+C	L+C	L+C	L+C	L+	L+C	L+C	C	L+C	L+C	L+C	C+L
2/4	C+L	L+C	3	L+C	C	C+L	3	C+	C+L	C+	C+L	L+C	C+	C+L
2/5	L+C	3	L+C	L+	C+L	C+	C+L	C+	C+L	C+	C+	C+	C+L	C+L
2/6	C+L	L+C	3	3	C	L+C	L+C	C+	L+C	C+L	C+L	L+C	L+C	C+
2/7	L+C	C+L	3	L+C	L+C	C	L+C	C+L	C+L	C+	L+C	L+C	L+C	C+L
2/8	C+L	C	3	L+C	C+	C	C	C+	L+C	C+	L+C	C	C	C+
2/9	C+	C+L	3	L+C	C+	C	C+	C	C	C+L	L+C	C+L	C+L	L+C
2/10	C+L	C	L+C	L+C	C+	L+C	L+	C+	3	C+	L+C	3	L+C	C+L
2/11	C+L	L+C	L+C	L+C	L+	L+C	3	C+	3	C+	L+C	3	C+L	C+L
2/12	C+	C+L	3	L+C	L+C	C+	C+	C	C+	C+	C+L	L+C	C+L	L+C
2/13	C+L	L+C	3	L+C	3	L+C	L+C	C+L	L+C	L+C	C+	L+C	L+C	C+L
2/14	C+L	C+L	L+C	L+C	C+	C+L	L+C	C+	C+	C+	C+L	C+	L+C	L+C
2/15	C+L	C+	3	L+C	L+C	C	L+C	C+L	L+C	C+	C+L	C	L+C	L+C
2/16	C+	C+	3	L+C	C+	C	C+L	C+	3	C+	C+L	*	L+C	C+L
2/17	C+L	C	3	L+C	C+	C	L+C	C+	C+L	C+	C+		C+L	L+C
2/18	C+L	C+	3	C+L	C+L	C+L	L+C	C+	C+	C	C+L		L+C	C+L
2/19	C+	C+	3	L+C	C+L	3	C+L	C	L+C	L+C	L+C		C	L+C
2/20	L+C	L+C	3	3	C+L	C+	C+	C	L+C	C+	L+C		C+L	C+L
2/21	C+	L+C	3	L+C	C+	C	C+	C+L	C+L	C	C+L		L+C	C+L
2/22	C+	L+C	L+C	L+C	L+C	C+L	L+N	C	3	C+L	L+C		3	C+
2/23	C	L+C	C+L	L+C	L+C	C	C+	C+L	L+C	C+	L+C		L+	C
2/24	L+C	C+L	L+	L+C	C+	C+L	C+L	L+C	L+C	C	L+C		3	L+C
2/25	L+	C+	3	L+C	C+	L+C	L+C	L+C	L+C	C+L	C+		L+C	C+L
2/26	3	C	3	L+C	C+L	C+L	C+L	C+	L+C	C+L	L+C		L+C	C+
2/27	L+C	C+	3	L+C	C+L	C+L	L+C	C+L	C+L	C+	L+C		L+C	L+C
2/28	L+C	L+	3	L+C	L+C	C+	L+C	C+L	C+L	C+L	C+L		C	C+L
2/29	L+C	L+C	3	L+	C+L	C	L+C	L+C	L+C	C+	C+		C	C+L

* Subject died

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BIOGRAPHICAL SKETCH

I weathered most of my elementary education in the suburban schools of Fremont, California. During my high school years a delightful 18-month sojourn with my family to Europe provided a memorable educational highlight. I subsequently entered the University of California at Berkeley, where the atmosphere was almost European, and graduated in 1975. In 1976-77 I spent a year in graduate school at the University of Texas before returning to Berkeley to work at the field station for Dr. Frank Beach for one final year. In 1978 I entered the University of Florida to work with Dr. Donald Dewsbury. After receiving a master's degree in 1983 and completing the research for this dissertation, I moved to the University of North Carolina at Wilmington, where I have been teaching since 1986.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald A. Dewsbury, Chairman
Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Marc N. Branch
Professor of Psychology

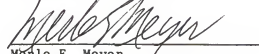
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. Jane Brockmann
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John F. Eisenberg
Katherine Ordway Professor of
Ecosystem Conservation

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Merle E. Meyer
Professor of Psychology

This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1989

Dean, Graduate School